Summary of Drinking Water Carcinogenicity Study of Methyl Acetoacetate in F344 Rats

September 2005

Japan Bioassay Research Center

Japan Industrial Safety and Health Association

PREFACE

The tests were contracted and supported by the Ministry of Health, Labour and Welfare of Japan. The tests were conducted by Japan Bioassay Research Center (JBRC) and the report was prepared by JBRC and peer reviewed by outside expert pathologist. Complete report was submitted to Ministry of Health, Labour and Welfare of Japan on September 28, 2005.

This English Summary was translated by JBRC from Japanese complete report.

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Purpose, materials and methods

Methyl acetoacetate (MAA, methyl 3-oxobutyrate, CAS No. 105-45-3) is a colorless clear liquid with a melting point of -80°C and a boiling point of 171.7°C, and is soluble in water (38 g/l00 mL water).

The carcinogenicity and chronic toxicity of MAA were examined in groups of 50 F344/DuCrj (Fischer) rats of both sexes administered MAA in drinking water for 2 years (104 weeks). The drinking water concentration of MAA was 0, 2000, 6325 or 20000 ppm (w/w). The highest dose level was chosen so as not to exceed the maximum tolerated dose (MTD), based on both growth rate and toxicity in the previous 13-week toxicity study. MAA was analyzed for purity and stability by both infrared spectrometry and gas chromatography before and after its use. The concentrations of MAA in drinking water were determined by gas chromatography at the time of preparation, and on the 8th day after preparation, while stored at room temperature. The animals were observed daily for clinical signs and mortality. Body weight, water consumption and food consumption were measured once a week for the first 14 weeks and every 4 weeks thereafter. Animals found dead, in a moribund state, or surviving to the end of the 2-year administration period underwent complete necropsy. Urinalysis was performed near the end of the administration period. For hematology and blood biochemistry, the surviving animals were bled under ether anesthesia, after they were fasted overnight, at the terminal necropsy. Organs and tissues were removed, weighed and examined for macroscopic lesions at necropsy. The organs and tissues were fixed and embedded in paraffin. Tissue sections of 5 μm thick were prepared and stained with hematoxylin and eosin and examined for histopathology. Incidences of neoplastic lesions were statistically analyzed by Fisher's exact test. A positive trend of dose-response relationship for the neoplastic incidence was analyzed by Peto's test. Incidences of non-neoplastic lesions and urinalysis were analyzed by Chi-square test. Changes in body weight, water consumption, food consumption, hematological and blood biochemical parameters, and organ weights were analyzed by Dunnett's test. The present studies were conducted in accordance with the Organisation for Economic Co-operation and Development (OECD) Good Laboratory Practice and with reference to the OECD Guideline for Testing of Chemicals 451 "Carcinogenicity Studies".

Results

There was no significant difference in survival rate between any MAA-administered male group and the male control. The survival rate of the 20000 ppm-administered female group was decreased during the middle period of 2-year administration and thereafter, and the decreased survival rate was attributed to the increased number of deaths due to the renal lesion. The red urine resulting from the renal lesion was observed in the females administered 6325 ppm and above during the latter half of the 2-year administration period. Body weights of the groups of both sexes administered 6325 ppm and above were decreased dose-dependently. Food consumption was also decreased dose-dependently in the males administered 6325 ppm and above and in all the MAA-administered female groups throughout the administration period. A dose-dependent decrease in water consumption was noted in all the MAA-administered groups of both sexes throughout the 2-year administration period.

No significant increase in the incidence of neoplastic lesions or tumor-related lesions was found in any MAA-administered group of either sex. In the kidney, the incidence of papillary necrosis was significantly increased in the males administered 20000 ppm and in all the MAA-administered female groups, and the incidences of papillary mineralization and urothelial hyperplasia in the pelvis were significantly increased in the females administered 6325 ppm and above. The increased relative kidney weight in all the MAA-administered female groups and the increased plasma level of urea nitrogen in the 20000 ppm-administered females, together with the increased incidence of positive occult blood in the females administered 6325 ppm and above, were observed, indicating adverse effects on the kidney.

Conclusions

In rats, there was no evidence of carcinogenic activity of MAA in males or females.

TABLES

TABLES (CONTINUED)

TABLE 1 SURVIVAL ANIMAL NUMBERS AND BODY WEIGHT CHANGES OF MALE RATS IN THE 2-YEAR DRINKING WATER STUDY OF METHYL ACETOACETATE

Week on Study	Control		2000 ppm			6325 ppm			20000 ppm		
	Av. Wt.	No. of Surviv. <50>	Av. Wt.	% of cont.	No. of Surviv. <50>	Av. Wt.	% of cont.	No. of Surviv. <50>	Av. Wt.	% of cont.	No. of Surviv. <50>
0	125 (50)	50 / 50	125 (50)	100	50 / 50	125 (50)	100	50 / 50	125 (50)	100	50 / 50
1	155 (50)	50 / 50	153 (50)	99	50 / 50	152 (50)	98	50 / 50	147 (50)	95	50 / 50
2	183 (50)	50 / 50	183 (50)	100	50 / 50	182 (50)	99	50 / 50	176 (50)	96	50 / 50
3	206 (50)	50 / 50	208 (50)	101	50 / 50	206 (50)	100	50 / 50	201 (50)	98	50 / 50
4	223 (50)	50 / 50	225 (50)	101	50 / 50	223 (50)	100	50 / 50	215 (50)	96	50 / 50
5	238 (50)	50 / 50	240 (50)	101	50 / 50	236 (50)	99	50 / 50	230 (50)	97	50 / 50
6	249 (50)	50 / 50	251 (50)	101	50 / 50	246 (50)	99	50 / 50	239 (50)	96	50 / 50
7	259 (50)	50 / 50	262 (50)	101	50 / 50	255 (50)	98	50 / 50	248 (50)	96	50 / 50
8	268 (50)	50 / 50	272 (50)	101	50 / 50	265 (50)	99	50 / 50	256 (50)	96	50 / 50
9	277 (50)	50 / 50	281 (50)	101	50 / 50	274 (50)	99	50 / 50	264 (50)	95	50 / 50
10	284 (50)	50 / 50	288 (50)	101	50 / 50	279 (50)	98	50 / 50	268 (50)	94	50 / 50
11	291 (50)	50 / 50	294 (50)	101	50 / 50	284 (50)	98	50 / 50	272 (50)	93	50 / 50
12	297 (50)	50 / 50	300 (50)	101	50 / 50	289 (50)	97	50 / 50	275 (50)	93	50 / 50
13	301 (50)	50 / 50	305 (50)	101	50 / 50	293 (50)	97	50 / 50	280 (50)	93	50 / 50
14	304 (50)	50 / 50	308 (50)	101	50 / 50	297 (50)	98	50 / 50	284 (50)	93	50 / 50
18	325 (50)	50 / 50	329 (50)	101	50 / 50	317 (50)	98	50 / 50	300 (50)	92	50 / 50
22	340 (50)	50 / 50	343 (50)	101	50 / 50	328 (50)	96	50 / 50	308 (50)	91	50 / 50
26	351 (50)	50 / 50	352 (50)	100	50 / 50	337 (50)	96	50 / 50	314 (50)	89	50 / 50
30	361 (50)	50 / 50	362 (50)	100	50 / 50	346 (50)	96	50 / 50	319 (50)	88	50 / 50
34	370 (50)	50 / 50	370 (50)	100	50 / 50	353 (50)	95	50 / 50	325 (50)	88	50 / 50
38	376 (50)	50 / 50	376 (49)	100	49 / 50	357 (49)	95	49 / 50	326 (50)	87	50 / 50
42	382 (50)	50 / 50	381 (49)	100	49 / 50	361 (49)	95	49 / 50	328 (49)	86	49 / 50
46	389 (50)	50 / 50	389 (49)	100	49 / 50	367 (49)	94	49 / 50	336 (47)	86	47 / 50
50	396 (50)	50 / 50	396 (49)	100	49 / 50	372 (49)	94	49 / 50	341 (46)	86	46 / 50
54	397 (50)	50 / 50	395 (48)	99	48 / 50	371 (49)	93	49 / 50	340 (46)	86	46 / 50
58	404 (50)	50 / 50	403 (48)	100	48 / 50	379 (49)	94	49 / 50	345 (46)	85	46 / 50
62	409 (50)	50 / 50	408 (48)	100	48 / 50	383 (49)	94	49 / 50	347 (45)	85	45 / 50
66	411 (50)	50 / 50	410 (48)	100	48 / 50	385 (49)	94	49 / 50	353 (44)	86	44 / 50
70	415 (48)	48 / 50	414 (47)	100	47 / 50	390 (49)	94	49 / 50	354 (44)	85	44 / 50
74	418 (47)	47 / 50	417 (46)	100	46 / 50	391 (49)	94	49 / 50	356 (44)	85	44 / 50
78	417 (46)	46 / 50	419 (46)	100	46 / 50	393 (49)	94	49 / 50	357 (44)	86	44 / 50
82	423 (45)	45 / 50	421 (46)	100	46 / 50	395 (49)	93	49 / 50	356 (44)	84	44 / 50
86	422 (45)	45 / 50	418 (46)	99	46 / 50	397 (47)	94	47 / 50	355 (44)	84	44 / 50
90	426 (44)	44 / 50	417 (46)	98	46 / 50	395 (46)	93	46 / 50	351 (43)	82	43 / 50
94	426 (42)	42 / 50	410 (45)	96	45 / 50	392 (46)	92	46 / 50	350 (42)	82	42 / 50
98	426 (40)	40 / 50	406 (44)	95	44 / 50	383 (43)	90	43 / 50	345 (42)	81	42 / 50
102	419 (39)	39 / 50	398 (42)	95	42 / 50	374 (41)	89	41 / 50	338 (41)	81	41 / 50
104	411 (38)	38 / 50	389 (41)	95	41 / 50	369 (40)	90	40 / 50	335 (39)	82	39 / 50

< > : No. of effective animals, ( ) : No. of measured animals, Av. Wt. : Averaged body weight (Unit : g).

TABLE 2 SURVIVAL ANIMAL NUMBERS AND BODY WEIGHT CHANGES OF FEMALE RATS IN THE 2-YEAR DRINKING WATER STUDY OF METHYL ACETOACETATE

Week on Study	Control		2000 ppm			6325 ppm			20000 ppm		
	Av. Wt.	No. of Surviv. <50>	Av. Wt.	% of cont.	No. of Surviv. <50>	Av. Wt.	% of cont.	No. of Surviv. <50>	Av. Wt.	% of cont.	No. of Surviv. <50>
0	98 (50)	50 / 50	98 (50)	100	50 / 50	98 (50)	100	50 / 50	98 (50)	100	50 / 50
1	114 (50)	50 / 50	112 (50)	98	50 / 50	111 (50)	97	50 / 50	107 (50)	94	50 / 50
2	124 (50)	50 / 50	123 (50)	99	50 / 50	124 (50)	100	50 / 50	120 (50)	97	50 / 50
3	134 (50)	50 / 50	133 (50)	99	50 / 50	133 (50)	99	50 / 50	130 (50)	97	50 / 50
4	139 (50)	50 / 50	140 (50)	101	50 / 50	141 (50)	101	50 / 50	136 (50)	98	50 / 50
5	146 (50)	50 / 50	146 (50)	100	50 / 50	147 (50)	101	50 / 50	142 (50)	97	50 / 50
6	149 (50)	50 / 50	150 (50)	101	50 / 50	151 (50)	101	50 / 50	146 (50)	98	50 / 50
7	153 (50)	50 / 50	154 (50)	101	50 / 50	154 (50)	101	50 / 50	149 (50)	97	50 / 50
8	156 (50)	50 / 50	157 (50)	101	50 / 50	157 (50)	101	50 / 50	151 (50)	97	50 / 50
9	160 (50)	50 / 50	160 (50)	100	50 / 50	161 (50)	101	50 / 50	154 (50)	96	50 / 50
10	163 (50)	50 / 50	163 (50)	100	50 / 50	164 (50)	101	50 / 50	157 (50)	96	50 / 50
11	166 (50)	50 / 50	166 (50)	100	50 / 50	166 (50)	100	50 / 50	158 (50)	95	50 / 50
12	168 (50)	50 / 50	168 (50)	100	50 / 50	167 (50)	99	50 / 50	160 (50)	95	50 / 50
13	169 (50)	50 / 50	169 (50)	100	50 / 50	168 (50)	99	50 / 50	161 (50)	95	50 / 50
14	171 (50)	50 / 50	171 (50)	100	50 / 50	170 (50)	99	50 / 50	164 (50)	96	50 / 50
18	177 (50)	50 / 50	176 (50)	99	50 / 50	174 (50)	98	50 / 50	165 (50)	93	50 / 50
22	184 (50)	50 / 50	183 (50)	99	50 / 50	181 (50)	98	50 / 50	172 (50)	93	50 / 50
26	191 (50)	50 / 50	188 (50)	98	50 / 50	187 (50)	98	50 / 50	176 (50)	92	50 / 50
30	195 (50)	50 / 50	193 (50)	99	50 / 50	191 (50)	98	50 / 50	180 (49)	92	49 / 50
34	200 (50)	50 / 50	197 (50)	99	50 / 50	194 (50)	97	50 / 50	181 (49)	91	49 / 50
38	202 (50)	50 / 50	198 (50)	98	50 / 50	195 (50)	97	50 / 50	181 (49)	90	49 / 50
42	206 (50)	50 / 50	203 (50)	99	50 / 50	198 (50)	96	50 / 50	183 (48)	89	48 / 50
46	210 (49)	49 / 50	206 (50)	98	50 / 50	201 (50)	96	50 / 50	184 (48)	88	48 / 50
50	215 (49)	49 / 50	210 (50)	98	50 / 50	205 (50)	95	50 / 50	188 (45)	87	45 / 50
54	220 (49)	49 / 50	213 (50)	97	50 / 50	206 (50)	94	50 / 50	188 (44)	85	44 / 50
58	224 (49)	49 / 50	218 (50)	97	50 / 50	209 (50)	93	50 / 50	190 (44)	85	44 / 50
62	230 (49)	49 / 50	222 (50)	97	50 / 50	213 (50)	93	50 / 50	192 (44)	83	44 / 50
66	233 (49)	49 / 50	226 (50)	97	50 / 50	215 (50)	92	50 / 50	195 (44)	84	44 / 50
70	239 (49)	49 / 50	231 (50)	97	50 / 50	219 (50)	92	50 / 50	195 (43)	82	43 / 50
74	246 (48)	48 / 50	235 (50)	96	50 / 50	225 (49)	91	49 / 50	197 (42)	80	42 / 50
78	248 (48)	48 / 50	237 (50)	96	50 / 50	223 (48)	90	48 / 50	199 (40)	80	40 / 50
82	253 (47)	47 / 50	241 (50)	95	50 / 50	228 (48)	90	48 / 50	205 (37)	81	37 / 50
86	254 (46)	46 / 50	244 (50)	96	50 / 50	230 (45)	91	45 / 50	200 (34)	79	34 / 50
90	258 (41)	41 / 50	249 (50)	97	50 / 50	232 (43)	90	43 / 50	200 (32)	78	32 / 50
94	262 (40)	40 / 50	252 (49)	96	49 / 50	234 (43)	89	43 / 50	201 (31)	77	31 / 50
98	267 (39)	39 / 50	257 (47)	96	47 / 50	238 (41)	89	41 / 50	203 (28)	76	28 / 50
102	267 (38)	38 / 50	256 (43)	96	43 / 50	238 (40)	89	40 / 50	203 (25)	76	25 / 50
104	267 (38)	38 / 50	256 (43)	96	43 / 50	237 (40)	89	40 / 50	204 (25)	76	25 / 50

< > : No. of effective animals, ( ) : No. of measured animals, Av. Wt. : Averaged body weight (Unit : g).

TABLE 3 FOOD CONSUMPTION CHANGES OF MALE RATS IN THE 2-YEAR DRINKING WATER STUDY OF METHYL ACETOACETATE

Week on Study	Control		2000 ppm			6325 ppm			20000 ppm		
	Av. FC.	No. of Surviv. <50>	Av. FC.	% of cont.	No. of Surviv. <50>	Av. FC.	% of cont.	No. of Surviv. <50>	Av. FC.	% of cont.	No. of Surviv. <50>
1	13.5 (50)	50 / 50	13.2 (50)	98	50 / 50	12.9 (50)	96	50 / 50	11.7 (50)	87	50 / 50
2	14.6 (50)	50 / 50	14.5 (50)	99	50 / 50	14.0 (50)	96	50 / 50	13.4 (50)	92	50 / 50
3	15.3 (50)	50 / 50	15.5 (50)	101	50 / 50	14.9 (50)	97	50 / 50	14.4 (49)	94	50 / 50
4	15.1 (50)	50 / 50	15.1 (50)	100	50 / 50	14.7 (50)	97	50 / 50	14.1 (50)	93	50 / 50
5	15.3 (50)	50 / 50	15.3 (50)	100	50 / 50	14.7 (50)	96	50 / 50	14.3 (50)	93	50 / 50
6	14.7 (50)	50 / 50	14.9 (50)	101	50 / 50	14.2 (50)	97	50 / 50	13.6 (50)	93	50 / 50
7	14.8 (50)	50 / 50	15.1 (50)	102	50 / 50	14.3 (50)	97	50 / 50	13.7 (50)	93	50 / 50
8	14.9 (50)	50 / 50	15.1 (50)	101	50 / 50	14.2 (50)	95	50 / 50	13.5 (50)	91	50 / 50
9	14.8 (50)	50 / 50	15.2 (50)	103	50 / 50	14.2 (50)	96	50 / 50	13.6 (48)	92	50 / 50
10	14.7 (50)	50 / 50	14.8 (50)	101	50 / 50	13.9 (50)	95	50 / 50	13.3 (50)	90	50 / 50
11	14.6 (50)	50 / 50	14.5 (50)	99	50 / 50	13.9 (50)	95	50 / 50	13.1 (50)	90	50 / 50
12	14.4 (50)	50 / 50	14.4 (50)	100	50 / 50	13.7 (50)	95	50 / 50	12.9 (50)	90	50 / 50
13	14.5 (50)	50 / 50	14.7 (50)	101	50 / 50	14.0 (50)	97	50 / 50	13.1 (50)	90	50 / 50
14	13.8 (50)	50 / 50	13.9 (50)	101	50 / 50	13.3 (50)	96	50 / 50	12.4 (50)	90	50 / 50
18	14.1 (50)	50 / 50	14.3 (50)	101	50 / 50	13.6 (50)	96	50 / 50	12.7 (50)	90	50 / 50
20	14.2 (50)	50 / 50	14.3 (50)	101	50 / 50	13.5 (50)	95	50 / 50	12.6 (50)	89	50 / 50
22	14.6 (50)	50 / 50	14.8 (50)	101	50 / 50	14.1 (50)	97	50 / 50	13.0 (50)	89	50 / 50
26	14.8 (50)	50 / 50	14.5 (50)	98	50 / 50	14.0 (50)	95	50 / 50	12.8 (50)	86	50 / 50
30	14.5 (50)	50 / 50	14.6 (50)	101	50 / 50	14.1 (50)	97	50 / 50	12.9 (50)	89	50 / 50
34	14.5 (50)	50 / 50	14.5 (50)	100	50 / 50	13.9 (50)	96	50 / 50	12.8 (50)	88	50 / 50
38	14.1 (50)	50 / 50	14.4 (49)	102	49 / 50	13.7 (49)	97	49 / 50	12.5 (50)	89	50 / 50
42	14.6 (50)	50 / 50	14.7 (49)	101	49 / 50	14.2 (49)	97	49 / 50	12.8 (49)	88	49 / 50
46	14.8 (50)	50 / 50	15.1 (49)	102	49 / 50	14.4 (49)	97	49 / 50	13.2 (47)	89	47 / 50
50	14.9 (50)	50 / 50	15.1 (49)	101	49 / 50	14.2 (49)	95	49 / 50	13.3 (46)	89	46 / 50
54	14.6 (50)	50 / 50	15.0 (48)	103	48 / 50	14.5 (49)	99	49 / 50	13.3 (46)	91	46 / 50
58	14.8 (50)	50 / 50	14.9 (48)	101	48 / 50	14.3 (49)	97	49 / 50	13.2 (46)	89	46 / 50
62	15.3 (50)	50 / 50	15.8 (48)	103	48 / 50	15.2 (49)	99	49 / 50	13.7 (45)	90	45 / 50
66	15.4 (50)	50 / 50	15.6 (48)	101	48 / 50	15.0 (49)	97	49 / 50	13.7 (44)	89	44 / 50
70	15.2 (48)	48 / 50	15.3 (47)	101	47 / 50	14.8 (49)	97	49 / 50	13.7 (44)	90	44 / 50
74	15.2 (47)	47 / 50	15.3 (46)	101	46 / 50	14.7 (49)	97	49 / 50	13.4 (44)	88	44 / 50
78	15.2 (46)	46 / 50	15.6 (46)	103	46 / 50	14.9 (49)	98	49 / 50	13.8 (44)	91	44 / 50
82	15.2 (45)	45 / 50	15.3 (46)	101	46 / 50	14.6 (49)	96	49 / 50	13.3 (44)	88	44 / 50
86	15.2 (45)	45 / 50	15.3 (46)	101	46 / 50	14.6 (47)	96	47 / 50	13.3 (44)	88	44 / 50
90	15.5 (44)	44 / 50	15.3 (46)	99	46 / 50	14.5 (46)	94	46 / 50	13.1 (43)	85	43 / 50
94	14.8 (42)	42 / 50	14.4 (45)	97	45 / 50	14.2 (46)	96	46 / 50	12.8 (42)	86	42 / 50
98	15.1 (40)	40 / 50	14.5 (44)	96	44 / 50	14.2 (43)	94	43 / 50	12.8 (41)	85	42 / 50
102	15.1 (38)	39 / 50	14.4 (42)	95	42 / 50	14.2 (41)	94	41 / 50	12.9 (41)	85	41 / 50
104	14.6 (38)	38 / 50	13.6 (41)	93	41 / 50	14.0 (40)	96	40 / 50	12.4 (39)	85	39 / 50

< > : No. of effective animals, ( ) : No. of measured animals, Av. FC. : Averaged food consumption (Unit : g).

TABLE 4 FOOD CONSUMPTION CHANGES OF FEMALE RATS IN THE 2-YEAR DRINKING WATER STUDY OF METHYL ACETOACETATE

Week on Study	Control		2000 ppm			6325 ppm			20000 ppm		
	Av. FC.	No. of Surviv.	Av. FC.	% of cont.	No. of Surviv.	Av. FC.	% of cont.	No. of Surviv.	Av. FC.	% of cont.	No. of Surviv.
	<50>		<50>			<50>			<50>		
1	10.3 (50)	50 / 50	9.9 (50)	96	50 / 50	9.5 (50)	92	50 / 50	8.8 (50)	85	50 / 50
2	10.3 (50)	50 / 50	9.9 (50)	96	50 / 50	9.8 (50)	95	50 / 50	9.4 (50)	91	50 / 50
3	10.3 (50)	50 / 50	10.0 (50)	97	50 / 50	9.9 (50)	96	50 / 50	9.3 (50)	90	50 / 50
4	10.1 (50)	50 / 50	9.8 (50)	97	50 / 50	9.6 (50)	95	50 / 50	9.2 (50)	91	50 / 50
5	10.2 (50)	50 / 50	10.0 (50)	98	50 / 50	9.8 (50)	96	50 / 50	9.2 (50)	90	50 / 50
6	9.8 (50)	50 / 50	9.6 (49)	98	50 / 50	9.5 (50)	97	50 / 50	8.9 (50)	91	50 / 50
7	9.6 (50)	50 / 50	9.4 (50)	98	50 / 50	9.1 (50)	95	50 / 50	8.9 (50)	93	50 / 50
8	9.6 (50)	50 / 50	9.3 (50)	97	50 / 50	9.1 (50)	95	50 / 50	8.6 (50)	90	50 / 50
9	9.8 (50)	50 / 50	9.5 (50)	97	50 / 50	9.4 (50)	96	50 / 50	8.8 (50)	90	50 / 50
10	9.8 (50)	50 / 50	9.6 (50)	98	50 / 50	9.3 (50)	95	50 / 50	8.7 (50)	89	50 / 50
11	9.8 (50)	50 / 50	9.5 (50)	97	50 / 50	9.2 (50)	94	50 / 50	8.7 (50)	89	50 / 50
12	9.7 (50)	50 / 50	9.5 (50)	98	50 / 50	9.1 (50)	94	50 / 50	8.6 (50)	89	50 / 50
13	10.0 (50)	50 / 50	9.7 (50)	97	50 / 50	9.5 (50)	95	50 / 50	8.8 (50)	88	50 / 50
14	9.8 (50)	50 / 50	9.6 (50)	98	50 / 50	9.1 (50)	93	50 / 50	8.6 (50)	88	50 / 50
18 a)	- (-)	50 / 50	- (-)	-	50 / 50	- (-)	-	50 / 50	- (-)	-	50 / 50
20	9.8 (50)	50 / 50	9.6 (50)	98	50 / 50	9.1 (50)	93	50 / 50	8.7 (50)	89	50 / 50
22	10.4 (50)	50 / 50	9.9 (50)	95	50 / 50	9.6 (50)	92	50 / 50	9.1 (50)	88	50 / 50
26	10.1 (50)	50 / 50	9.9 (50)	98	50 / 50	9.5 (50)	94	50 / 50	8.9 (50)	88	50 / 50
30	10.1 (50)	50 / 50	9.9 (50)	98	50 / 50	9.5 (50)	94	50 / 50	8.9 (49)	88	49 / 50
34	10.1 (50)	50 / 50	9.9 (50)	98	50 / 50	9.4 (50)	93	50 / 50	8.8 (49)	87	49 / 50
38	10.1 (50)	50 / 50	9.7 (50)	96	50 / 50	9.3 (50)	92	50 / 50	8.8 (49)	87	49 / 50
42	10.4 (50)	50 / 50	10.3 (50)	99	50 / 50	9.8 (50)	94	50 / 50	8.9 (48)	86	48 / 50
46	10.4 (49)	49 / 50	10.1 (50)	97	50 / 50	9.8 (50)	94	50 / 50	9.2 (48)	88	48 / 50
50	10.6 (49)	49 / 50	10.3 (50)	97	50 / 50	9.9 (50)	93	50 / 50	9.0 (45)	85	45 / 50
54	10.8 (49)	49 / 50	10.5 (50)	97	50 / 50	10.0 (50)	93	50 / 50	9.3 (44)	86	44 / 50
58	11.0 (49)	49 / 50	10.7 (50)	97	50 / 50	10.1 (50)	92	50 / 50	9.4 (44)	85	44 / 50
62	10.9 (49)	49 / 50	10.5 (50)	96	50 / 50	10.0 (50)	92	50 / 50	9.1 (44)	83	44 / 50
66	10.8 (49)	49 / 50	10.6 (50)	98	50 / 50	10.0 (50)	93	50 / 50	9.2 (44)	85	44 / 50
70	11.4 (49)	49 / 50	10.9 (50)	96	50 / 50	10.3 (50)	90	50 / 50	9.4 (43)	82	43 / 50
74	11.1 (48)	48 / 50	10.6 (50)	95	50 / 50	10.3 (49)	93	49 / 50	9.2 (42)	83	42 / 50
78	11.4 (48)	48 / 50	10.5 (50)	92	50 / 50	10.0 (48)	88	48 / 50	9.6 (40)	84	40 / 50
82	11.2 (47)	47 / 50	10.8 (50)	96	50 / 50	10.3 (48)	92	48 / 50	9.9 (37)	88	37 / 50
86	11.2 (46)	46 / 50	11.0 (50)	98	50 / 50	10.5 (45)	94	45 / 50	9.8 (34)	88	34 / 50
90	11.8 (41)	41 / 50	11.3 (50)	96	50 / 50	10.8 (43)	92	43 / 50	9.7 (32)	82	32 / 50
94	11.5 (40)	40 / 50	10.9 (49)	95	49 / 50	10.4 (43)	90	43 / 50	9.6 (31)	83	31 / 50
98	11.9 (39)	39 / 50	11.5 (47)	97	47 / 50	11.0 (41)	92	41 / 50	9.7 (28)	82	28 / 50
102	11.6 (38)	38 / 50	11.2 (43)	97	43 / 50	10.5 (40)	91	40 / 50	9.9 (25)	85	25 / 50
104	12.0 (38)	38 / 50	11.3 (43)	94	43 / 50	10.7 (40)	89	40 / 50	10.1 (25)	84	25 / 50

< > : No. of effective animals, ( ) : No. of measured animals, Av. FC. : Averaged food consumption (Unit : g).
a) : Food consumption could not be calculated because the measurement of food consumption failed.

TABLE 5 WATER CONSUMPTION CHANGES OF MALE RATS IN THE 2-YEAR DRINKING WATER STUDY OF METHYL ACETOACETATE

Week on Study	Control		2000 ppm			6325 ppm			20000 ppm		
	Av. WC.	No. of Surviv. <50>	Av. WC.	% of cont.	No. of Surviv. <50>	Av. WC.	% of cont.	No. of Surviv. <50>	Av. WC.	% of cont.	No. of Surviv. <50>
1	17.7 (50)	50 / 50	14.6 (50)	82	50 / 50	13.5 (50)	76	50 / 50	14.2 (50)	80	50 / 50
2	19.2 (50)	50 / 50	15.7 (50)	82	50 / 50	14.3 (50)	74	50 / 50	13.4 (50)	70	50 / 50
3	19.0 (50)	50 / 50	16.5 (50)	87	50 / 50	15.4 (50)	81	50 / 50	14.2 (50)	75	50 / 50
4	18.0 (50)	50 / 50	15.9 (50)	88	50 / 50	14.2 (50)	79	50 / 50	12.9 (46)	72	50 / 50
5	18.8 (50)	50 / 50	16.7 (50)	89	50 / 50	15.9 (50)	85	50 / 50	13.9 (50)	74	50 / 50
6	19.1 (50)	50 / 50	16.7 (50)	87	50 / 50	15.9 (50)	83	50 / 50	13.7 (50)	72	50 / 50
7	19.5 (50)	50 / 50	16.8 (50)	86	50 / 50	15.2 (50)	78	50 / 50	13.1 (50)	67	50 / 50
8	19.1 (50)	50 / 50	16.2 (50)	85	50 / 50	14.5 (50)	76	50 / 50	12.2 (50)	64	50 / 50
9	18.7 (50)	50 / 50	16.0 (50)	86	50 / 50	13.8 (50)	74	50 / 50	11.8 (50)	63	50 / 50
10	18.5 (50)	50 / 50	15.4 (50)	83	50 / 50	13.2 (50)	71	50 / 50	11.0 (50)	59	50 / 50
11	17.8 (50)	50 / 50	14.8 (50)	83	50 / 50	13.1 (50)	74	50 / 50	10.8 (50)	61	50 / 50
12	17.1 (50)	50 / 50	14.3 (50)	84	50 / 50	12.8 (50)	75	50 / 50	10.2 (50)	60	50 / 50
13	18.7 (50)	50 / 50	16.4 (50)	88	50 / 50	13.0 (50)	70	50 / 50	12.4 (50)	66	50 / 50
14	18.5 (50)	50 / 50	15.0 (50)	81	50 / 50	12.7 (50)	69	50 / 50	11.4 (50)	62	50 / 50
18	17.0 (50)	50 / 50	15.1 (50)	89	50 / 50	11.5 (50)	68	50 / 50	10.1 (50)	59	50 / 50
22	17.5 (50)	50 / 50	14.6 (50)	83	50 / 50	12.0 (50)	69	50 / 50	10.4 (50)	59	50 / 50
26	17.0 (50)	50 / 50	14.7 (50)	86	50 / 50	12.1 (50)	71	50 / 50	10.8 (50)	64	50 / 50
30	17.0 (50)	50 / 50	15.3 (50)	90	50 / 50	12.4 (50)	73	50 / 50	10.6 (49)	62	50 / 50
34	17.8 (49)	50 / 50	15.4 (50)	87	50 / 50	12.7 (50)	71	50 / 50	10.5 (49)	59	50 / 50
38	17.0 (50)	50 / 50	14.9 (49)	88	49 / 50	12.1 (49)	71	49 / 50	11.1 (50)	65	50 / 50
42	17.9 (50)	50 / 50	15.7 (49)	88	49 / 50	13.2 (49)	74	49 / 50	11.4 (49)	64	49 / 50
46	16.9 (50)	50 / 50	15.1 (49)	89	49 / 50	12.2 (49)	72	49 / 50	9.6 (47)	57	47 / 50
50	17.2 (50)	50 / 50	15.4 (49)	90	49 / 50	12.6 (49)	73	49 / 50	10.5 (46)	61	46 / 50
54	15.4 (50)	50 / 50	13.4 (48)	87	48 / 50	11.0 (49)	71	49 / 50	9.4 (46)	61	46 / 50
58	16.7 (50)	50 / 50	14.8 (48)	89	48 / 50	12.4 (49)	74	49 / 50	10.3 (46)	62	46 / 50
62	17.8 (50)	50 / 50	15.1 (48)	85	48 / 50	12.3 (49)	69	49 / 50	10.0 (45)	56	45 / 50
66	17.2 (50)	50 / 50	15.7 (48)	91	48 / 50	12.3 (49)	72	49 / 50	10.5 (44)	61	44 / 50
70	17.1 (48)	48 / 50	15.2 (47)	89	47 / 50	12.5 (49)	73	49 / 50	9.9 (44)	58	44 / 50
74	17.4 (47)	47 / 50	15.4 (46)	89	46 / 50	13.2 (49)	76	49 / 50	10.8 (44)	62	44 / 50
78	17.8 (46)	46 / 50	16.3 (46)	92	46 / 50	14.1 (49)	79	49 / 50	11.6 (44)	65	44 / 50
82	18.0 (45)	45 / 50	16.7 (46)	93	46 / 50	14.6 (49)	81	49 / 50	11.6 (44)	64	44 / 50
86	18.4 (45)	45 / 50	16.7 (46)	91	46 / 50	13.8 (47)	75	47 / 50	11.7 (44)	64	44 / 50
90	19.7 (44)	44 / 50	18.6 (46)	94	46 / 50	14.7 (46)	75	46 / 50	12.3 (43)	62	43 / 50
94	19.0 (42)	42 / 50	16.4 (45)	86	45 / 50	14.2 (46)	75	46 / 50	12.5 (42)	66	42 / 50
98	18.9 (40)	40 / 50	15.7 (44)	83	44 / 50	13.5 (43)	71	43 / 50	11.3 (42)	60	42 / 50
102	20.2 (39)	39 / 50	16.4 (42)	81	42 / 50	14.9 (41)	74	41 / 50	13.0 (41)	64	41 / 50
104	20.4 (38)	38 / 50	16.2 (41)	79	41 / 50	14.7 (40)	72	40 / 50	12.0 (39)	59	39 / 50

< > : No. of effective animals, ( ) : No. of measured animals, Av. WC. : Averaged water consumption (Unit : g).

TABLE 6 WATER CONSUMPTION CHANGES OF FEMALE RATS IN THE 2-YEAR DRINKING WATER STUDY OF METHYL ACETOACETATE

Week on Study	Control		2000 ppm			6325 ppm			20000 ppm		
	Av. WC.	No. of Surviv. <50>	Av. WC.	% of cont.	No. of Surviv. <50>	Av. WC.	% of cont.	No. of Surviv. <50>	Av. WC.	% of cont.	No. of Surviv. <50>
1	15.9 (50)	50 / 50	12.2 (50)	77	50 / 50	11.2 (49)	70	50 / 50	10.6 (50)	67	50 / 50
2	17.6 (50)	50 / 50	12.4 (50)	70	50 / 50	11.3 (50)	64	50 / 50	10.7 (50)	61	50 / 50
3	16.8 (50)	50 / 50	12.2 (50)	73	50 / 50	11.1 (50)	66	50 / 50	10.1 (50)	60	50 / 50
4	17.9 (49)	50 / 50	12.6 (50)	70	50 / 50	10.9 (50)	61	50 / 50	9.8 (50)	55	50 / 50
5	18.5 (50)	50 / 50	12.3 (50)	66	50 / 50	10.8 (50)	58	50 / 50	9.6 (50)	52	50 / 50
6	17.8 (50)	50 / 50	12.3 (50)	69	50 / 50	10.7 (50)	60	50 / 50	9.9 (50)	56	50 / 50
7	17.9 (47)	50 / 50	12.1 (50)	68	50 / 50	10.2 (50)	57	50 / 50	9.6 (50)	54	50 / 50
8	17.8 (47)	50 / 50	12.4 (50)	70	50 / 50	10.0 (50)	56	50 / 50	8.7 (50)	49	50 / 50
9	17.6 (48)	50 / 50	11.8 (50)	67	50 / 50	10.0 (50)	57	50 / 50	10.1 (50)	57	50 / 50
10	18.6 (46)	50 / 50	12.3 (50)	66	50 / 50	10.1 (50)	54	50 / 50	10.4 (50)	56	50 / 50
11	20.7 (48)	50 / 50	12.0 (50)	58	50 / 50	9.6 (50)	46	50 / 50	9.4 (50)	45	50 / 50
12	17.7 (49)	50 / 50	11.4 (50)	64	50 / 50	9.1 (50)	51	50 / 50	8.5 (50)	48	50 / 50
13	19.3 (44)	50 / 50	12.0 (50)	62	50 / 50	9.9 (50)	51	50 / 50	8.2 (50)	42	50 / 50
14	18.8 (47)	50 / 50	11.8 (50)	63	50 / 50	9.7 (50)	52	50 / 50	8.2 (50)	44	50 / 50
18	16.3 (49)	50 / 50	9.9 (49)	61	50 / 50	7.4 (50)	45	50 / 50	6.3 (50)	39	50 / 50
22	19.9 (47)	50 / 50	12.2 (48)	61	50 / 50	9.9 (50)	50	50 / 50	8.6 (50)	43	50 / 50
26	17.8 (49)	50 / 50	12.2 (50)	69	50 / 50	9.4 (50)	53	50 / 50	8.2 (50)	46	50 / 50
30	18.6 (49)	50 / 50	12.0 (49)	65	50 / 50	9.3 (50)	50	50 / 50	8.2 (49)	44	49 / 50
34	17.6 (49)	50 / 50	12.3 (50)	70	50 / 50	10.2 (50)	58	50 / 50	8.7 (49)	49	49 / 50
38	17.1 (48)	50 / 50	11.6 (50)	68	50 / 50	9.2 (50)	54	50 / 50	8.6 (49)	50	49 / 50
42	17.5 (49)	50 / 50	13.3 (50)	76	50 / 50	10.3 (50)	59	50 / 50	8.8 (48)	50	48 / 50
46	15.7 (48)	49 / 50	10.9 (50)	69	50 / 50	9.8 (50)	62	50 / 50	8.0 (48)	51	48 / 50
50	16.3 (48)	49 / 50	12.5 (50)	77	50 / 50	10.0 (50)	61	50 / 50	8.5 (45)	52	45 / 50
54	15.4 (48)	49 / 50	12.8 (50)	83	50 / 50	10.0 (50)	65	50 / 50	8.5 (44)	55	44 / 50
58	15.0 (47)	49 / 50	11.8 (50)	79	50 / 50	9.9 (50)	66	50 / 50	8.4 (44)	56	44 / 50
62	15.3 (48)	49 / 50	11.3 (50)	74	50 / 50	9.5 (50)	62	50 / 50	8.1 (44)	53	44 / 50
66	16.1 (49)	49 / 50	11.2 (50)	70	50 / 50	9.8 (50)	61	50 / 50	8.8 (44)	55	44 / 50
70	15.7 (48)	49 / 50	11.3 (50)	72	50 / 50	10.0 (50)	64	50 / 50	9.2 (43)	59	43 / 50
74	14.7 (48)	48 / 50	11.6 (50)	79	50 / 50	10.7 (49)	73	49 / 50	9.2 (42)	63	42 / 50
78	15.2 (48)	48 / 50	11.4 (50)	75	50 / 50	10.7 (48)	70	48 / 50	10.2 (40)	67	40 / 50
82	15.3 (46)	47 / 50	12.0 (50)	78	50 / 50	11.4 (48)	75	48 / 50	11.2 (37)	73	37 / 50
86	14.9 (46)	46 / 50	12.0 (50)	81	50 / 50	11.6 (45)	78	45 / 50	10.9 (34)	73	34 / 50
90	16.2 (41)	41 / 50	13.1 (50)	81	50 / 50	12.3 (43)	76	43 / 50	11.5 (32)	71	32 / 50
94	17.0 (40)	40 / 50	12.3 (49)	72	49 / 50	11.7 (43)	69	43 / 50	11.6 (31)	68	31 / 50
98	16.8 (39)	39 / 50	12.8 (47)	76	47 / 50	13.0 (41)	77	41 / 50	12.6 (28)	75	28 / 50
102	15.9 (38)	38 / 50	13.4 (43)	84	43 / 50	13.6 (40)	86	40 / 50	12.3 (25)	77	25 / 50
104	17.2 (36)	38 / 50	14.0 (43)	81	43 / 50	14.1 (40)	82	40 / 50	12.9 (25)	75	25 / 50

< > : No. of effective animals, ( ) : No. of measured animals, Av. WC. : Averaged water consumption (Unit : g).

TABLE 7 HEMATOLOGY OF MALE RATS IN THE 2-YEAR DRINKING WATER STUDY OF METHYL ACETOACETATE

Group Name	Control	2000 ppm	6325 ppm	20000 ppm
No. of examined animals	38	39	40	38
HEMOGLOBIN (g/dL)	14.4 ± 1.7	14.2 ± 2.4	14.3 ± 2.7	15.2 ± 1.8 *
PLATELET ($10^3/\mu$ L)	848 ± 266	820 ± 264	799 ± 308	705 ± 139 **

Mean ± S.D.
Significant difference: * : p≦0.05 ** : p≦0.01 Test of Dunnett

TABLE 8 HEMATOLOGY OF FEMALE RATS IN THE 2-YEAR DRINKING WATER STUDY OF METHYL ACETOACETATE

Group Name	Control	2000 ppm	6325 ppm	20000 ppm
No. of examined animals	38	43	40	24
RED BLOOD CELL ($10^6/\mu$ L)	8.19 ± 0.59	7.73 ± 1.13 *	7.86 ± 1.22 **	7.80 ± 0.56 **
HEMOGLOBIN (g/dL)	14.9 ± 1.1	14.2 ± 1.9 *	14.5 ± 2.1 *	14.2 ± 0.8 **
HEMATOCRIT (%)	43.1 ± 2.7	41.1 ± 4.8	42.0 ± 6.4 *	41.4 ± 2.2 **
PLATELET ($10^3/\mu$ L)	634 ± 144	612 ± 136	674 ± 166	679 ± 82 *

Mean ± S.D.
Significant difference: * : p≦0.05 ** : p≦0.01 Test of Dunnett

TABLE 9 BIOCHEMISTRY OF MALE RATS IN THE 2-YEAR DRINKING WATER STUDY OF METHYL ACETOACETATE

Group Name	Control	2000 ppm	6325 ppm		20000 ppm	
No. of examined animals	38	41	40		38	
TOTAL PROTEIN (g/dL)	6.7 ± 0.5	6.6 ± 0.4	6.6 ± 0.3		6.5 ± 0.4	**
A/G RATIO	0.8 ± 0.1	0.8 ± 0.1	0.8 ± 0.1	*	0.9 ± 0.1	**
T-CHOLESTEROL (mg/dL)	181 ± 53	160 ± 38	151 ± 39	**	145 ± 41	**
TRIGLYCERIDE (mg/dL)	82 ± 51	74 ± 48	71 ± 43		50 ± 35	**
PHOSPHOLIPID (mg/dL)	255 ± 75	234 ± 81	219 ± 54	*	209 ± 60	**
LDH (IU/L)	188 ± 75	554 ± 2138	218 ± 351		156 ± 78	**
ALP (IU/L)	203 ± 58	265 ± 266	264 ± 106	**	225 ± 60	
POTASSIUM (mEq/L)	3.4 ± 0.2	3.6 ± 0.5	3.6 ± 0.3	*	3.5 ± 0.3	*

Mean ± S.D.
Significant difference: * : p≦0.05 ** : p≦0.01 Test of Dunnett

TABLE 10 BIOCHEMISTRY OF FEMALE RATS IN THE 2-YEAR DRINKING WATER STUDY OF METHYL ACETOACETATE

Group Name	Control	2000 ppm	6325 ppm		20000 ppm	
No. of examined animals	38	43	40		24	
TOTAL PROTEIN (g/dL)	6.9 ± 0.4	6.9 ± 0.5	6.8 ± 0.5		6.4 ± 0.5	**
A/G RATIO	1.0 ± 0.1	1.0 ± 0.1	1.0 ± 0.2		1.1 ± 0.1	*
AST (IU/L)	161 ± 112	197 ± 208	120 ± 63		109 ± 95	**
ALT (IU/L)	70 ± 44	65 ± 38	46 ± 15	*	44 ± 23	**
LDH (IU/L)	215 ± 62	378 ± 618	238 ± 147		176 ± 79	*
UREA NITROGEN (mg/dL)	16.7 ± 1.8	16.4 ± 1.7	17.9 ± 3.3		18.4 ± 3.2	*
INORGANIC PHOSPHORUS (mg/dL)	3.8 ± 0.7	4.1 ± 0.7	4.3 ± 0.6	**	4.4 ± 0.7	**

Mean ± S.D.
Significant difference: * : p≦0.05 ** : p≦0.01 Test of Dunnett

TABLE 11 URINALYSIS OF MALE RATS IN THE 2-YEAR DRINKING WATER STUDY OF METHYL ACETOACETATE

Group Name		Control	2000 ppm	6325 ppm	20000 ppm
No. of examined animals		38	41	40	39
	Grade				
pH	5.0	0	0	0	0
	6.0	1	2	0	1
	6.5	5	11	4	18
	7.0	18	18	14	7
	7.5	11	8	12	11
	8.0	3	2	9	2
	8.5	0	0	1	0
	Chi square test				*
Protein	−	0	0	0	0
	±	0	0	0	0
	+	0	1	1	1
	2+	12	3	6	7
	3+	22	30	26	27
	4+	4	7	7	4
	Chi square test		*		
Ketone body	−	34	29	32	26
	±	4	11	6	13
	+	0	1	2	0
	2+	0	0	0	0
	3+	0	0	0	0
	4+	0	0	0	0
	Chi square test				*

Significant difference: * : $p \leqq 0.05$ ** : $p \leqq 0.01$

TABLE 12 URINALYSIS OF FEMALE RATS IN THE 2-YEAR DRINKING WATER STUDY OF METHYL ACETOACETATE

Group Name		Control	2000 ppm	6325 ppm	20000 ppm
No. of examined animals		38	43	40	25
	Grade				
pH	5.0	0	0	0	0
	6.0	0	0	1	6
	6.5	2	7	13	8
	7.0	5	8	11	4
	7.5	7	12	6	3
	8.0	20	15	6	3
	8.5	4	1	3	1
	Chi square test			**	**
Protein	−	0	0	0	0
	±	1	0	1	0
	+	15	4	0	1
	2+	11	16	15	3
	3+	7	18	17	14
	4+	4	5	7	7
	Chi square test		*	**	**
Occult blood	−	36	37	19	1
	±	0	1	1	0
	+	1	0	1	0
	2+	0	1	1	0
	3+	1	4	18	24
	Chi square test			**	**

Significant difference: * : $p \leqq 0.05$ ** : $p \leqq 0.01$

TABLE 13 ORGAN WEIGHTS OF MALE RATS IN THE 2-YEAR DRINKING WATER STUDY OF METHYL ACETOACETATE

Group Name	Control	2000 ppm		6325 ppm		20000 ppm	
No. of examined animals	38	41		40		39	
Body weight (g)	388 ± 32	366 ± 35		349 ± 20	**	319 ± 31	**
Adrenals (g)	0.069 ± 0.010	0.094 ± 0.184		0.064 ± 0.018	**	0.061 ± 0.024	**
Adrenals (%)	0.018 ± 0.002	0.027 ± 0.054		0.018 ± 0.005		0.019 ± 0.008	
Heart (g)	1.193 ± 0.098	1.159 ± 0.083		1.135 ± 0.118	*	1.044 ± 0.086	**
Heart (%)	0.309 ± 0.026	0.319 ± 0.031		0.326 ± 0.036	*	0.329 ± 0.031	*
Lungs (g)	1.350 ± 0.073	1.473 ± 0.405		1.349 ± 0.210		1.238 ± 0.112	**
Lungs (%)	0.350 ± 0.027	0.412 ± 0.163	**	0.389 ± 0.072	**	0.391 ± 0.047	**
Kidneys (g)	2.571 ± 0.225	2.653 ± 0.298		2.497 ± 0.127		2.407 ± 0.217	**
Kidneys (%)	0.665 ± 0.060	0.735 ± 0.146	**	0.718 ± 0.045	**	0.759 ± 0.085	**
Spleen (g)	0.995 ± 0.435	1.193 ± 1.345		1.210 ± 1.357		0.818 ± 0.537	**
Spleen (%)	0.257 ± 0.113	0.336 ± 0.431		0.355 ± 0.434		0.257 ± 0.181	
Liver (g)	10.396 ± 1.253	10.038 ± 1.383		9.514 ± 0.957	*	8.405 ± 0.949	**
Liver (%)	2.682 ± 0.232	2.751 ± 0.353		2.737 ± 0.316		2.640 ± 0.238	
Brain (g)	2.053 ± 0.043	2.057 ± 0.049		2.049 ± 0.038		2.029 ± 0.045	*
Brain (%)	0.533 ± 0.044	0.568 ± 0.062		0.590 ± 0.037	**	0.643 ± 0.081	**

Mean ± S.D.
Significant difference: * : $p \leqq 0.05$ ** : $p \leqq 0.01$ Test of Dunnett

TABLE 14 ORGAN WEIGHTS OF FEMALE RATS IN THE 2-YEAR DRINKING WATER STUDY OF METHYL ACETOACETATE

Group Name	Control	2000 ppm		6325 ppm		20000 ppm	
No. of examined animals	38	43		40		25	
Body weight (g)	250 ± 21	240 ± 24		223 ± 21	**	191 ± 19	**
Adrenals (g)	0.066 ± 0.008	0.147 ± 0.507		0.065 ± 0.015		0.067 ± 0.033	**
Adrenals (%)	0.027 ± 0.004	0.063 ± 0.218		0.030 ± 0.008	*	0.035 ± 0.016	**
Ovaries (g)	0.114 ± 0.023	0.142 ± 0.188		0.347 ± 1.511		0.106 ± 0.021	
Ovaries (%)	0.046 ± 0.011	0.057 ± 0.060		0.158 ± 0.690	**	0.056 ± 0.014	**
Heart (g)	0.855 ± 0.057	0.840 ± 0.056		0.813 ± 0.079	**	0.783 ± 0.104	**
Heart (%)	0.344 ± 0.027	0.353 ± 0.038		0.367 ± 0.039	*	0.416 ± 0.094	**
Lungs (g)	0.995 ± 0.065	1.015 ± 0.183		1.024 ± 0.304	*	0.944 ± 0.117	**
Lungs (%)	0.401 ± 0.039	0.427 ± 0.084		0.462 ± 0.139	**	0.502 ± 0.104	**
Kidneys (g)	1.709 ± 0.135	1.739 ± 0.146		1.793 ± 0.238		1.707 ± 0.156	
Kidneys (%)	0.687 ± 0.056	0.729 ± 0.070	*	0.807 ± 0.085	**	0.901 ± 0.105	**
Spleen (g)	0.573 ± 0.176	1.013 ± 1.458		0.717 ± 0.732		0.523 ± 0.394	**
Spleen (%)	0.231 ± 0.072	0.426 ± 0.599		0.320 ± 0.310		0.287 ± 0.278	
Liver (g)	6.480 ± 0.809	6.571 ± 1.491		6.062 ± 1.059	*	5.508 ± 0.845	**
Liver (%)	2.605 ± 0.324	2.736 ± 0.523		2.717 ± 0.326		2.900 ± 0.417	**
Brain (g)	1.894 ± 0.039	1.880 ± 0.038		1.863 ± 0.038	**	1.840 ± 0.048	**
Brain (%)	0.764 ± 0.069	0.792 ± 0.087		0.843 ± 0.073	**	0.975 ± 0.110	**

Mean ± S.D.
Significant difference: * : $p \leqq 0.05$ ** : $p \leqq 0.01$ Test of Dunnett

TABLE 15 INCIDENCES OF SELECTED NEOPLASTIC LESIONS OF MALE RATS IN THE 2-YEAR DRINKING WATER STUDY OF METHYL ACETOACETATE

Group Name	Control	2000 ppm	6325 ppm	20000 ppm	Peto test	Cochran-Armitage test
Number of examined animals	50	50	50	50		
thyroid	<50>	<50>	<50>	<50>		
C-cell adenoma	12 (24 %)	6 (12 %)	14 (28 %)	4 (8 %) *		

Significant difference * : p≦0.05 ** : p≦0.01 Fisher's exact test for neoplastic lesion
↑(↓) : p≦0.05 ↑↑(↓↓) : p≦0.01 Peto or Cochran-Armitage test for neoplastic lesion
< > : Number of animals examined at the site

TABLE 16 INCIDENCES OF SELECTED NEOPLASTIC LESIONS OF FEMALE RATS IN THE 2-YEAR DRINKING WATER STUDY OF METHYL ACETOACETATE

Group Name	Control	2000 ppm	6325 ppm	20000 ppm	Peto test	Cochran-Armitage test
Number of examined animals	50	50	50	50		
spleen	<50>	<50>	<50>	<50>		
mononuclear cell leukemia	3 (6 %)	5 (10 %)	7 (14 %)	6 (12 %)	↑	
uterus	<50>	<50>	<50>	<50>		
endometrial stromal polyp	10 (20 %)	7 (14 %)	5 (10 %)	12 (24 %)	↑	

Significant difference * : p≦0.05 ** : p≦0.01 Fisher's exact test for neoplastic lesion
↑(↓) : p≦0.05 ↑↑(↓↓) : p≦0.01 Peto or Cochran-Armitage test for neoplastic lesion
< > : Number of animals examined at the site

TABLE 17 INCIDENCES OF SELECTED NON-NEOPLASTIC LESIONS OF MALE RATS IN THE 2-YEAR DRINKING WATER STUDY OF METHYL ACETOACETATE

Group Name	Control				2000 ppm				6325 ppm				20000 ppm			
Number of examined animals	50				50				50				50			
Grade of non-neoplastic lesion	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
liver	<50>				<50>				<50>				<50>			
acidophilic cell focus	11	1	1	0	8	2	0	0	9	0	2	0	2	0	0	0 *
kidney	<50>				<50>				<50>				<50>			
chronic nephropathy	27	11	3	0	33	12	1	0	47	0	0	0 **	38	3	0	0 *
papillary necrosis	0	1	0	0	1	2	0	0	1	0	0	0	10	1	0	0 **
thyroid	<50>				<50>				<50>				<50>			
C-cell hyperplasia	16	0	0	0	16	0	0	0	9	0	0	0	5	0	0	0 *
eye	<50>				<50>				<50>				<50>			
retinal atrophy	24	21	4	0	23	13	9	0	28	9	8	0 *	34	8	6	0 *

Grade 1: Slight 2 : Moderate 3 : Marked 4 :Severe
< > : Number of animals examined at the site
Significant difference ; * : p ≦ 0.05 ** : p ≦ 0.01 Test of Chi Square

TABLE 18 INCIDENCES OF SELECTED NON-NEOPLASTIC LESIONS OF FEMALE RATS IN THE 2-YEAR DRINKING WATER STUDY OF METHYL ACETOACETATE

Group Name	Control				2000 ppm				6325 ppm				20000 ppm			
Number of examined animals	50				50				50				50			
Grade of non-neoplastic lesion	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
nasal cavity	<50>				<50>				<50>				<50>			
mineralization	17	0	0	0	17	0	0	0	17	0	0	0	7	0	0	0 *
liver	<50>				<50>				<50>				<50>			
granulation	31	4	0	0	21	5	0	0	17	1	0	0 **	19	2	0	0 *
kidney	<50>				<50>				<50>				<50>			
papillary necrosis	0	0	0	0	19	1	0	0 **	18	11	0	0 **	21	19	0	0 **
mineralization:papilla	0	0	0	0	0	0	0	0	3	8	0	0 **	4	15	0	0 **
urothelial hyperplasia:pelvis	0	0	0	0	0	0	0	0	2	2	1	0	5	3	0	0 *
eye	<50>				<50>				<50>				<50>			
retinal atrophy	11	35	3	0	20	28	2	0	37	10	0	0 **	32	11	6	0 **
adrenal	<50>				<50>				<50>				<50>			
peliosis-like lesion	33	3	0	0	36	5	0	0	32	5	0	0	23	1	0	0 *
nasolacrimal duct	<50>				<50>				<50>				<50>			
inflammation	7	2	0	0	2	1	0	0	3	1	0	0	1	0	0	0 *

Grade 1: Slight 2 : Moderate 3 : Marked 4 :Severe
< > : Number of animals examined at the site
Significant difference ; * : p ≦ 0.05 ** : p ≦ 0.01 Test of Chi Square

TABLE 19 HISTORICAL CONTROL DATA OF SELECTED NEOPLASTIC LESIONS IN JAPAN BIOASSAY RESEARCH CENTER : F344/DuCrj FEMALE RATS

Organs Tumors	No. of animals examined	No. of animals bearing tumor	Incidence (%)	Min. - Max. (%)
Uterus	1696			
Endometrial stromal polyp		246	14.5	2 - 28
Spleen	1697			
Mononuclear cell leukemia		222	13.1	2 - 26

34 carcinogenicity studies examined in Japan Bioassay Research Center were used.
Study No. : 0043, 0059, 0061, 0063, 0065, 0067, 0095, 0104, 0115, 0130, 0141, 0158, 0162, 0189, 0205, 0210, 0224, 0242, 0267, 0269, 0278, 0284, 0296, 0303, 0318, 0328, 0342, 0347, 0365, 0371, 0399, 0401, 0417, 0421

TABLE 20 CAUSE OF DEATH OF RATS IN THE 2-YEAR DRINKING WATER STUDY OF METHYL ACETOACETATE

	Male				Female			
Group name	Control	2000 ppm	6325 ppm	20000 ppm	Control	2000 ppm	6325 ppm	20000 ppm
Number of dead or moribund animals	12	9	10	11	12	7	10	25
No microscopical confirmation	0	0	0	2	0	0	1	2
Urinary system lesion	0	1	0	0	0	0	0	0
Renal lesion	0	1	1	1	0	0	0	8
Urinary retention	1	1	0	0	0	0	0	0
Deglutition disorder	0	0	0	2	0	0	1	2
Tumor death ： leukemia	1	2	3	1	2	1	4	5
skin/appendage	0	0	1	0	0	0	0	0
subcutis	1	1	1	1	0	0	0	1
nasal cavity	0	0	0	0	0	1	0	0
oral cavity	0	0	0	0	1	0	1	0
tooth	0	1	0	0	0	0	1	0
large intestine	0	0	0	0	0	1	0	1
liver	0	0	0	1	0	0	0	0
pituitary gland	3	0	1	2	3	1	1	2
thyroid	1	0	0	0	0	0	0	0
adrenal gland	0	1	1	0	0	1	0	0
uterus	—	—	—	—	2	0	0	1
mammary gland	0	0	0	0	1	2	0	2
preputial/clitoral gland	2	0	0	0	0	0	0	1
brain	2	0	0	0	0	0	1	0
spinal cord	1	0	0	0	0	0	0	0
muscle	0	0	1	0	0	0	0	0
bone	0	0	1	0	2	0	0	0
vertebra	0	0	0	1	0	0	0	0
mediastinum	0	0	0	0	1	0	0	0
retroperitoneum	0	1	0	0	0	0	0	0

FIGURES

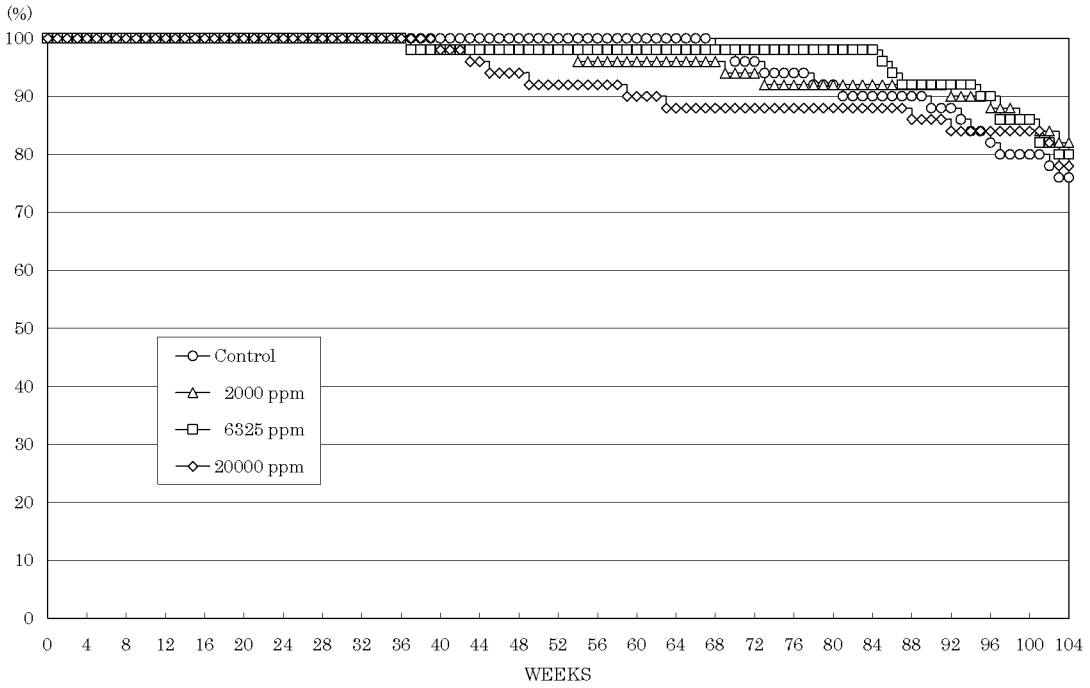


FIGURE 1 SURVIVAL ANIMAL RATE OF MALE RATS IN THE 2-YEAR DRINKING WATER STUDY OF METHYL ACETOACETATE

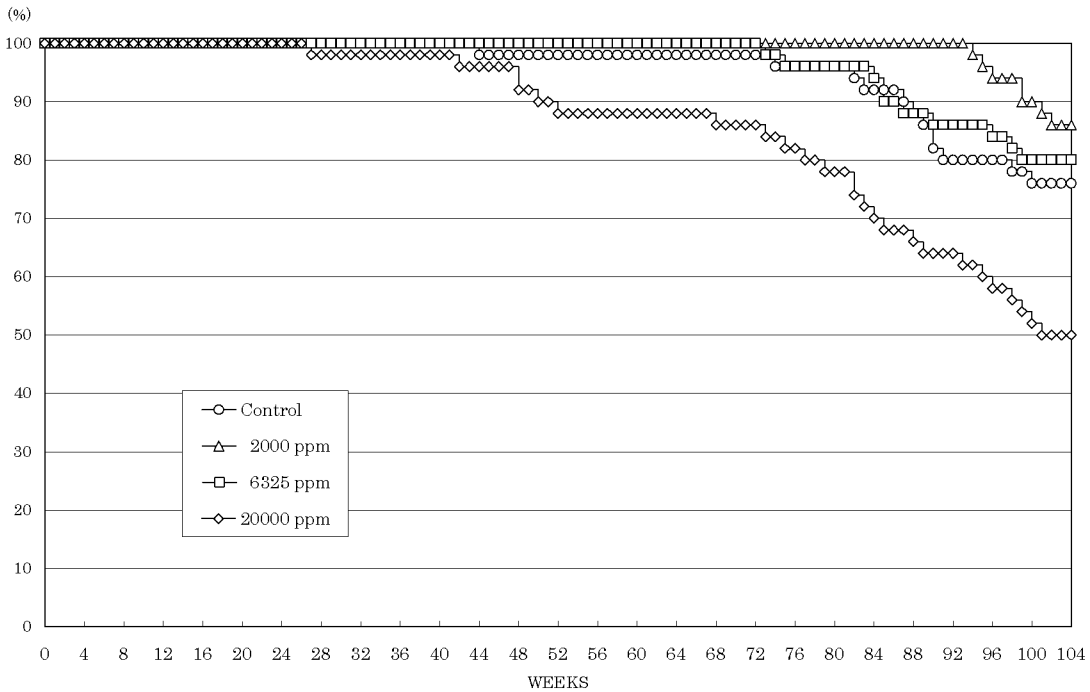


FIGURE 2 SURVIVAL ANIMAL RATE OF FEMALE RATS IN THE 2-YEAR DRINKING WATER STUDY OF METHYL ACETOACETATE

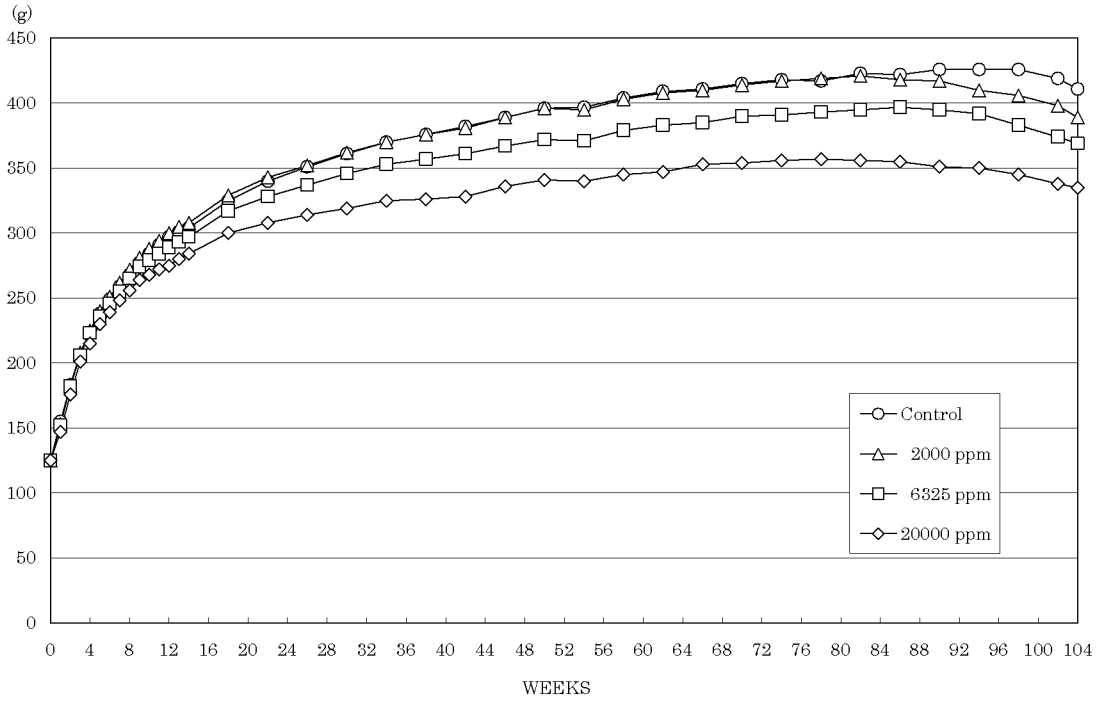


FIGURE 3 BODY WEIGHT CHANGES OF MALE RATS IN THE 2-YEAR DRINKING WATER STUDY OF METHYL ACETOACETATE

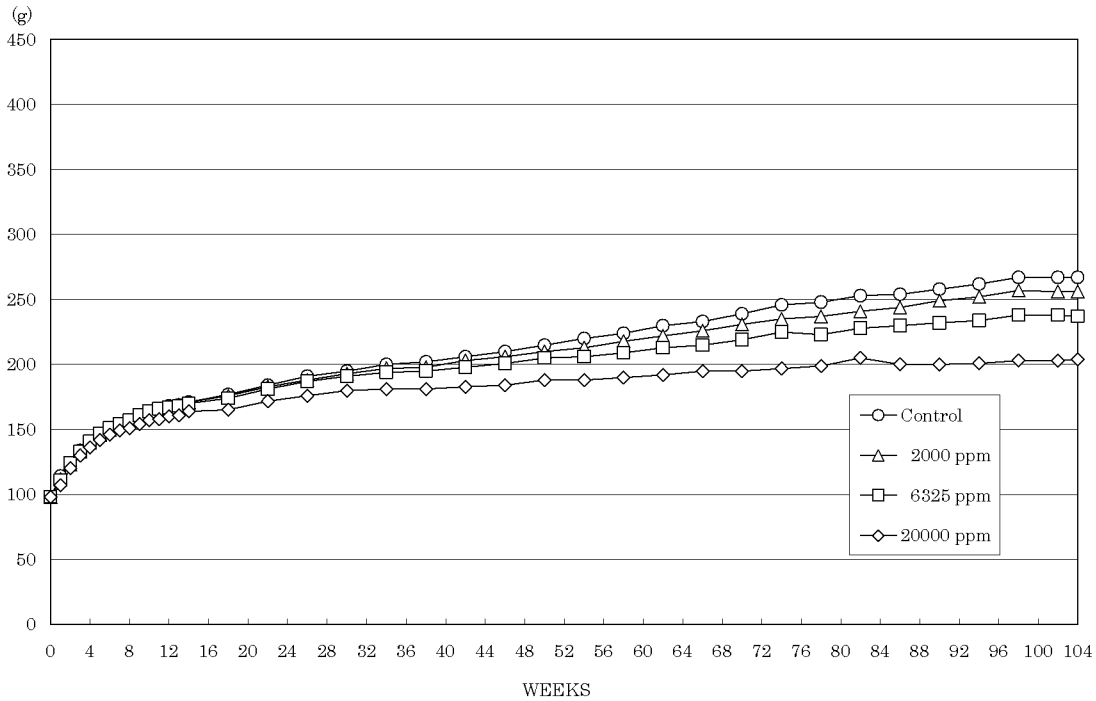


FIGURE 4 BODY WEIGHT CHANGES OF FEMALE RATS IN THE 2-YEAR DRINKING WATER STUDY OF METHYL ACETOACETATE

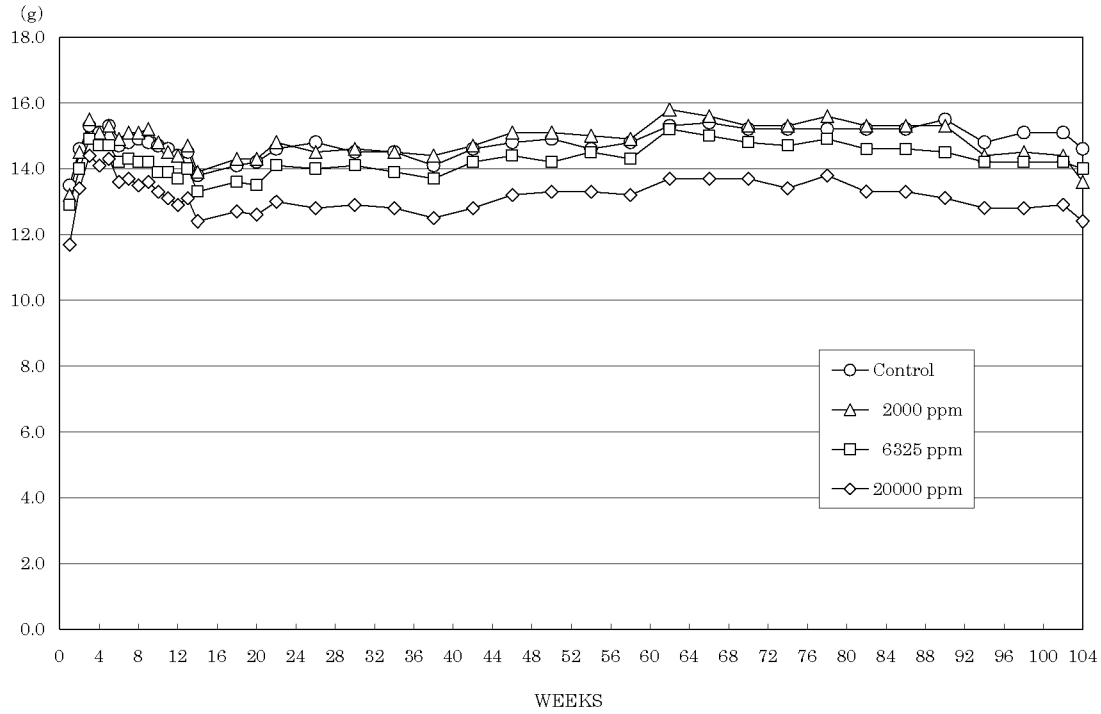


FIGURE 5 FOOD CONSUMPTION CHANGES OF MALE RATS IN THE 2-YEAR DRINKING WATER STUDY OF METHYL ACETOACETATE

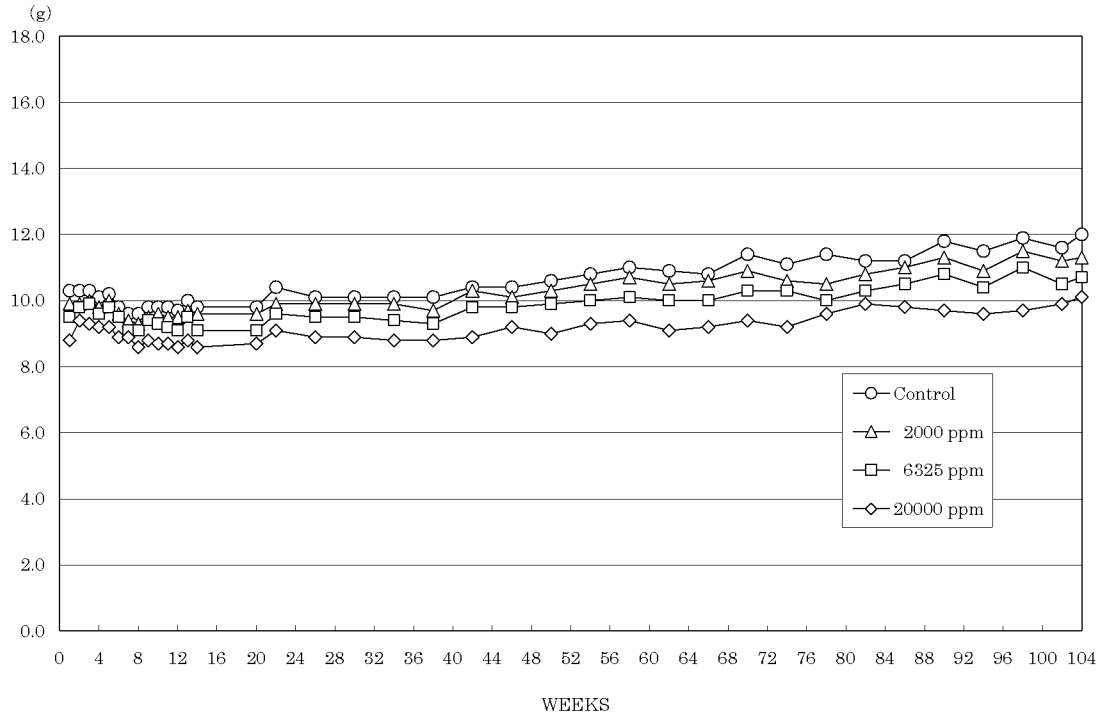


FIGURE 6 FOOD CONSUMPTION CHANGES OF FEMALE RATS IN THE 2-YEAR DRINKING WATER STUDY OF METHYL ACETOACETATE

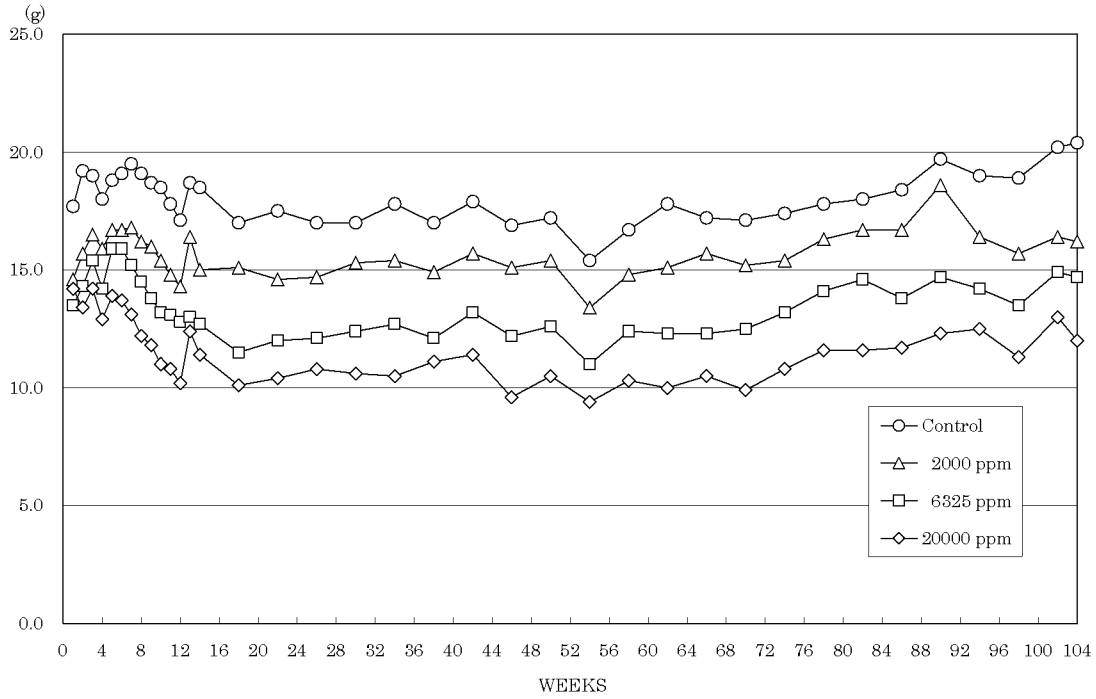


FIGURE 7 WATER CONSUMPTION CHANGES OF MALE RATS IN THE 2-YEAR DRINKING WATER STUDY OF METHYL ACETOACETATE

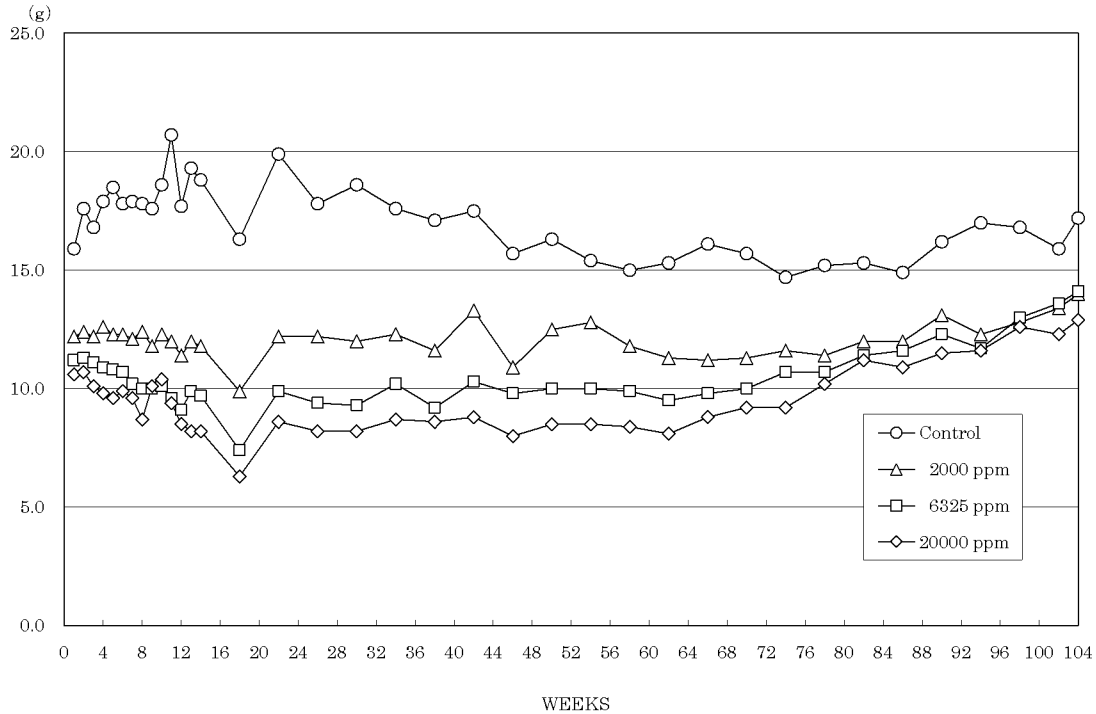
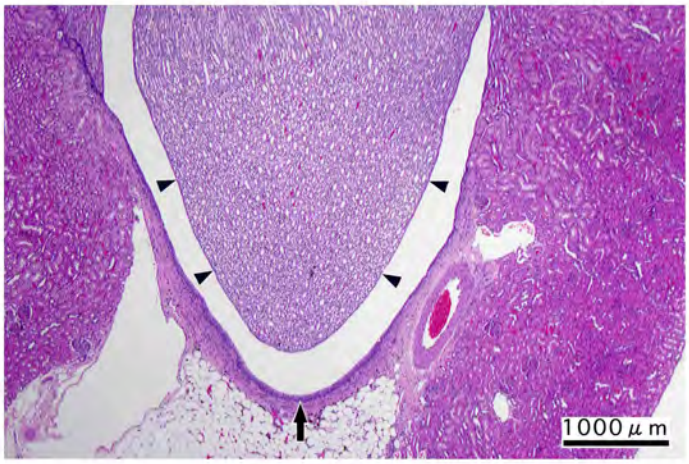
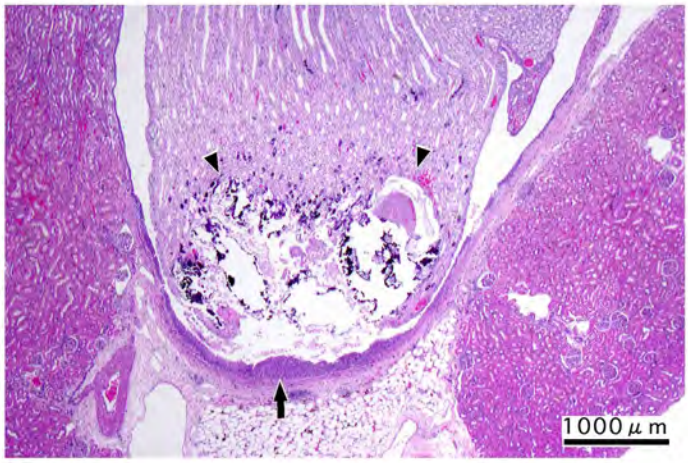


FIGURE 8 WATER CONSUMPTION CHANGES OF FEMALE RATS IN THE 2-YEAR DRINKING WATER STUDY OF METHYL ACETOACETATE



Photograph 1
Kidney: Normal
Papilla (Arrow-heads)
Urothelium of the pelvis (Arrow)
Rat, Female, Control, Animal No. 0448-2001 (H&E)



Photograph 2
Kidney
Papillary necrosis (Arrow-heads)
Urothelial hyperplasia of the pelvis (Arrow)
Rat, Female, 20000 ppm, Animal No. 0448-2306 (H&E)